

300 Rec'd PCT/PTO 29 SEP 1998

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INSTANT VESICULAR PRODUCT

The invention relates to an instant vesicular product, a process for the preparation thereof and compositions comprising the said product.

Vesicles in apolar vehicles were described in 1991 by H. Kunieda et al (J. Am. Chem. Soc. 113 (3) 1051-1052). The reversed vesicles, consisting essentially of the hydrophilic surfactant tetra-ethyleneglycol dodecyl ether in dodecane, were found to coalesce and revert back to a lamellar liquid crystalline phase over a period of hours to days, despite the addition of about 2.5 water molecules per ethyleneoxide-unit. Further publications on the same issue and by the same authors disclosed a preference for the use of straight chain hydrocarbon compounds as the apolar medium (H. Kunieda et al: Langmuir 1991 (7) 1915-1919, J. Coll. Interf. Sci 1991 (147) 286-288, J. Coll. Interf. Sci 1993 (156) 446-453). However, such dispersions have a very limited practical significance for product development in view of the very bad cosmetic and palatability properties of the said compounds, their toxic properties and also because these compounds are not biodegradable. International patent application WO 93/00069 disclosed dispersions of reversed vesicles in apolar vehicles, which vesicles were stable during a considerable period of time. The dispersions of vesicles disclosed therein were prepared by sonicating a mixture consisting of one or more surfactants, a lipophilic stabilising factor, optionally a hydrophilic stabilising factor and an apolar vehicle. The components for preparing the reversed vesicles in principle can be selected from a variety of materials. However, on substituting the hydrocarbon compounds by biodegradable apolar compounds, such as glycerol tri-esters of higher saturated and unsaturated fatty acids having 10-30 carbon atoms and vegetable oils, the yield of reversed vesicles, as assessed by polarised-light microscopy, is rather poor in the present inventors' experience, as compared to the yield when such vesicles are prepared in a hydrocarbon vehicle.

Since dispersions of reversed vesicles have shown distinct advantages over those of vesicles in aqueous vehicles, among other things a high encapsulating capacity for both lipophilic and hydrophilic drugs and a high encapsulating efficiency for hydrophilic drugs, there has been a demand to find a way to increase the yield of reversed vesicles in apolar pharmaceutically and cosmetically acceptable vehicles, such as the above-mentioned glycerol tri-esters and vegetable oils, without adversely affecting the encapsulating capacity and efficiency thereof. International patent application WO 95/20945 discloses a dispersion of

reversed vesicles, which was prepared from specially processed galactolipids, obtained from oat kernels, in a MCT oil by sonication for 1 hour at 30-40°C. The presence of large reversed vesicles was assessed by means of a differential interference phase contrast microscope, but neither an assessment of the amount of vesicles formed was made nor a particle size distribution provided. The dispersions were said to be stable for about one week.

It has now been found that by making a primary dispersion of reversed vesicles in a suitable apolar vehicle and subsequently removing the said apolar vehicle a powder of reversed vesicles is obtained, which on dispersion in the same apolar vehicle maintains its vesicular structure and thus the dispersion of reversed vesicles is instantaneously obtained again. Surprisingly the same powder of reversed vesicles on dispersion in another apolar vehicle, such as a biodegradable oil, also maintains its vesicular structure and in this way a secondary dispersion of reversed vesicles is instantaneously obtained. The amount of reversed vesicles in the biodegradable oil appears to be very high as compared with the yield of reversed vesicles when these would have been prepared directly in the biodegradable oil.

The powder of reversed vesicles comprises one or more non-ionic surfactants and optionally a lipophilic stabilising factor, such as cholesterol. Other examples of compounds to be used as the lipophilic stabilising factor can be found in WO 93/00069. The product may further comprise a bio-active agent. The non-ionic surfactant is advantageously a derivative of a pentose, a hexose or an oligomer thereof, such as a fatty acid ester or a fatty alcohol ether. By preference the non-ionic surfactant is a fatty acid ester of a pentose, such as xylose, a hexose, such as glucose, fructose, galactose, mannose or maltitol, or an oligomer thereof, such as sucrose, lactose or lactulose. Since the pentoses and hexoses avail of more than one esterifiable hydroxy-group, the fatty acid esters of these compounds consist of a mixture of mono-, di-, tri- and poly-esters. Most preferably those products are used which contain at least 50 wt% of mono-esters and there is even more preference for those compounds, containing at least 70 wt% of mono-esters, the percentages based on the weight of the surfactant. The same applies to the corresponding ether compounds. Suitable fatty acids for the esterification are C8-C30 straight chain saturated and unsaturated fatty acids, such as lauric acid, myristic acid, palmitic acid, stearic acid, behenic acid and oleic acid. In particular and also in view of the commercial availability thereof fatty acid esters of sucrose are used for the preparation of the instant product. Suitable examples thereof are S-970 (sucrose stearate, 50% mono ester, 50% di-, tri- and poly ester); P-1570 (sucrose palmitate, 70% mono

ester); S-1670 (sucrose stearate, 75% mono ester); M-1695 (sucrose myristate, 80% mono ester) and L-1695 (sucrose laurate, 80% mono ester).

The powder of reversed vesicles is prepared by a process, which comprises the steps of:

- making a primary dispersion of reversed vesicles by sonication or microfluidisation of a mixture consisting of one or more non-ionic surfactants and optionally a lipophilic stabilising factor and a bio-active agent in a suitable apolar vehicle and
- subsequently removing the said apolar vehicle.

The reversed vesicles and/or the components, making up the vesicles, including the bio-active agent, are insoluble or practically insoluble in the apolar vehicle to be used for making the primary dispersion of reversed vesicles. The apolar vehicle is selected from compounds or mixtures thereof which, when evaporation techniques will be used, preferably have a high vapour pressure, in particular below the temperature at which the vesicles melt. Examples of such apolar vehicles are volatile silicone oils, such as Abil® K4, isoalkanes, such as isoparaffines, and (C1-C4)-alkyl alkanoates, such as ethyl acetate.

The primary dispersion of reversed vesicles may be prepared according to methods known in the art, e.g. such as disclosed in international patent application WO 93/00069. Preferably during the preparation of the primary dispersion of reversed vesicles a hydrophilic stabilising factor, such as water, is added. It appeared that small amounts thereof are sufficient to reduce the particle size of the vesicles and, as the result thereof, to increase the amount of reversed vesicles and the rate at which the reversed vesicles are formed. E.g. in case a sucrose ester is used as the non-ionic surfactant, an amount of up to 15 wt% of water, the percentage based on the weight of the surfactant, is advantageously used. More preferably 5-10 wt% of water is added during the preparation of the primary dispersion of reversed vesicles. As it appears to be the case, the water can be added at several stages during the preparation, but preferably it is present right at the beginning.

Removal of the apolar vehicle from the primary dispersion of reversed vesicles can be performed in several ways, such as by evaporation, centrifugation, filtration, lyophilisation etc. However, it is important that the bilayer structure of the vesicles will not be perturbed during the removal. There is a preference for evaporation techniques, in particular rotational evaporation and spray-drying. On using these processes it has appeared that the addition of excipients, such as the so-called cryoprotectants used during lyophilisation processes, is not necessary.

The product, obtained as described above and in details in the appended examples, consists of a vesicular structure, as a consequence of which a bio-active agent, if included in the primary dispersion of reversed vesicles, remains encapsulated. It may together with one or more excipients be incorporated in compositions, encompassing another aspect of the invention. The excipients may be solid in the form of dry powders or granulates in order to make tablets, capsules etc. The excipients may also be liquid or semi-solid in order to prepare dispersions. The liquid may be a polar compound, such as water or propylene glycol, or is a biodegradable compound. It has been demonstrated that in this way it is possible to make dispersions of reversed vesicles in a biodegradable apolar compound in a high amount, as compared to those directly made in the biodegradable compound according to the methods known in the art. Examples of such biodegradable natural or synthetic compounds are fatty acids, such as oleic acid, vegetable oils, such as peanut-oil and sesame oil, and mono-, di- and triglycerides of saturated and unsaturated, straight-chain fatty acids with 12 to 30 carbon atoms such as lauric acid, myristic acid, palmitic acid, stearic acid and arachidonic acid.

Whichever the way the product according to the present invention is incorporated into a composition, it is clear that the bad cosmetic and palatability properties of the non-volatile hydrocarbon apolar dispersion vehicle have been eliminated. Since the encapsulation efficiency of the reversed vesicles for bio-active agents is highly influenced by the choice of the apolar vehicle, it is a further advantage that the product according to the present invention is obtained using an apolar vehicle, which is a non-solvent, preferably also for the bio-active agent. On dispersion of the product in another apolar solvent to instantaneously obtain a secondary dispersion of reversed vesicles a high encapsulation efficiency of the bio-active agent has been found. Another advantage of the powder of reversed vesicles is the increase of stability of the various components and especially due to the structural integrity the prevention of leakage of bio-active compounds from the vesicles.

Although the foregoing invention has been described in some detail by way of illustration and example for purpose of clarity and understanding, it will be readily apparent to those of ordinary skill in the art in the light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit and the scope of the appended claims.

The following examples further illustrate the invention.

EXAMPLE 1

44.375 g of a silicone oil (Abil® K4), 5 g of sucrose palmitate P1570 (a mixture of
5 70% mono-esterified and 30% di- and poly-esterified sucrose, as obtained from C.N. Schmidt
B.V., Amsterdam, The Netherlands), 0.5 g of cholesterol and 0.125 g p-aminobenzoic acid
(PABA) were weighed into a thermostated vessel at 90°C. The mixture was sonified at 97
watts output during 30 minutes using a Branson sonifier 250 (Branson Ultrasonics Corp.
Danbury, U.S.A.) followed by cooling of the vessel with cooling water of 7.5°C during 15
10 minutes until room temperature was reached. During cooling the mixture was sonified at 97
watts output with a duty cycle of 50%. Crystallisation of the silicone oil at the wall of the
vessel was prevented by also stirring the mixture using a magnetic bar during cooling.

1.1 Removal of apolar vehicle by rotational evaporation

15 50 ml of the dispersion of vesicles so obtained was transferred to a 250 ml round
bottom flask. The silicone oil was allowed to evaporate using a Büchi Rotavap (Büchi Labo-
ratoriums AG, Flawil, Switzerland), the waterbath being kept at 30°C. The rotational speed of
the round bottom flask was set at five and the pressure was reduced to 0.1 bar. After
evaporation was completed, the remaining film was gathered and milled in a mortar.

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1.2 Removal of apolar vehicle by spray drying

50 ml of the dispersion of vesicles was transferred to a mini spray dryer (Büchi 190,
Büchi Laboratories AG Flawil, Switzerland), operating conditions: airflow 500 NL/h, inlet
temperature 67°C, outlet temperature 56°C.

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EXAMPLE 2

890 g of the silicone oil Abil® K4, 100 g of sucrose palmitate P1570 and 10 g of
cholesterol were weighed in a thermostated vessel kept at 70°C. The components were mixed
30 for ten minutes using an Ultra Turrax high shear mixer. After this pre-homogenisation the
mixture was transferred to a M110 T Microfluidiser device operated at a pressure of 9000 PSI
(Microfluidics Corp., Newton, U.S.A.) and several cycles were passed. The microfluidiser
was thermostated at 30°C using a Neslab Exacal Ex-410 device (Neslab, Newington, U.S.A.).

After the last cycle the dispersion was cooled using a Neslab Endocal RTE 220 flow-through cooling device (Neslab, Newington, U.S.A.), the temperature of the waterbath being set at 25°C.

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2.1 Removal of apolar vehicle by rotational evaporation

50 ml of the dispersion of vesicles so obtained was transferred to a 250 ml round bottom flask. The silicone oil was allowed to evaporate using a Büchi Rotavap (Büchi Laboratories AG, Flawil, Switzerland), the waterbath being kept at 30°C. The rotational speed of the round bottom flask was set at five and the pressure was reduced to 0.1 bar. After evaporation was completed, the remaining film was gathered and milled in a mortar.

2.2 Removal of apolar vehicle by spray drying

50 ml of the dispersion of vesicles was transferred to a mini spray dryer (Büchi 190, Büchi Laboratories AG Flawil, Switzerland), operating conditions: airflow 500 NL/h, inlet temperature 67°C, outlet temperature 56°C.

EXAMPLE 3

1.125 g of the powdered product obtained according to example 1.1 and 8.875 g of an oil, selected from the group consisting of caprylic/capric triglyceride (Miglyol® 812N), peanut oil, castor oil, oleic acid and the volatile silicone oil Abil® K4, were weighed in a 20 ml sample vial. The mixture was stirred for 10 minutes at 150 rpm using a magnetic stirrer. The presence of reversed vesicles in the dispersions as so-called Maltese crosses was assessed by polarised light microscopy (Olympus® BH2 Tokyo Japan) immediately after preparation and after storage of the dispersions for 2 weeks at room temperature. The results have been listed in table 1.

Table 1

powder dispersed in	appearance
Miglyol® 812N	reversed vesicles and a lot of agglomerates of reversed vesicles
Peanut-oil	reversed vesicles and a lot of agglomerates of reversed vesicles
Castor oil	reversed vesicles and a lot of agglomerates of reversed vesicles
Oleic acid	reversed vesicles and some agglomerates of reversed vesicles
Abil® K4	reversed vesicles and a lot of agglomerates of reversed vesicles

- 5 No change in the appearance was observed after storage during two weeks at room temperature.

EXAMPLE 4

- 10 1.125 g of the powdered product obtained according to example 1.1 and 8.875 g of an oil, selected from the group consisting of caprylic/capric triglyceride (Miglyol® 812N), peanut oil, castor oil and the volatile silicone oil Abil® K4, were weighed in a 20 ml sample vial. The mixture was stirred for 10 minutes at 150 rpm using a magnetic stirrer.

- 15 Directly after preparation the encapsulation efficiency of PABA, defined as the percentage PABA encapsulated per gram of reversed vesicles dispersion, was calculated by means of the formula:

$$EF = [1 - (f \cdot FP/TP)] \cdot 100\%$$

wherein: f = the weight fraction of non-encapsulated apolar vehicle

- 20 FP = the concentration (mg/g) of PABA dissolved in the non-encapsulated apolar vehicle

TP = the concentration (mg/g) of PABA dissolved in the dispersion of reversed vesicles in the apolar vehicle.

The results have been listed in table 2.

Table 2

Powder dispersed in:	Encapsulation efficiency (%) mean \pm SD (n=3)
Miglyol® 812N	71.8 \pm 14.2
peanut oil	71.2 \pm 0.9
castor oil	57.9 \pm 0.8
Abil® K4	98.5 \pm 0.1

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EXAMPLE 5

A dispersion of reversed vesicles in an apolar medium, which is caprylic/capric tri-glyceride (Miglyol® 812N), peanut oil, castor oil, oleic acid and the silicone oil Abil® K4 was made according to the method of example 1.

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The appearance of the dispersions was assessed by means of a polarised-light microscope as described in example 3, immediately after preparation and after storage of the dispersions for two weeks at room temperature. The results have been listed in table 3.

Table 3

dispersion of reversed vesicles directly prepared in	appearance
Miglyol® 812N	some giant reversed vesicles and a lot of non-vesicular material
Peanut-oil	some reversed vesicles, some PABA crystals and a lot of non-vesicular material
Castor oil	non-vesicular material only
Oleic acid	non-vesicular material only
Abil® K4	reversed vesicles only

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No change in the appearance was observed after storage during two weeks at room temperature.

The encapsulation efficiency of PABA was not determined, due to the lack of (sufficient) vesicular material.

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EXAMPLE 6

The silicone oil Abil® K4, sucrose palmitate P-1570, cholesterol and water in the amounts as indicated in table 4, were weighed into a thermostated reaction vessel of 100 ml at 88°C. The mixture was sonicated using a Branson Sonifier 250 equipped with a 4.8 mm diameter microtip at 88 Watts output for 30 minutes. Subsequently, the sample was cooled to ambient temperature using a cooling bath at 15°C. Cooling was performed for 20 minutes under stirring to prevent agglomeration.

Two batches were prepared, wherein the water was added 15 and 30 minutes respectively after sonication had started.

All batches were stirred in sealed glass vials at room temperature.

On dispersing the powder of reversed vesicles in Abil® K4 it was observed that the vesicular structure was not changed due to the removal of the apolar vehicle.

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Table 4

sucrose palmitate	10	10	10	10	10	10	10
cholesterol	1	1	1	1	1	1	1
silicone oil	89	86,5	84	79	79	79	74
water content (%)	0	2,5	5	10	10 after 15 min	10 after 30 min	15
particle size (microscopial)	6-12 µm	2-6 µm	1-4 µm	1-4 µm	1-4 µm	8-16 µm	1-4 µm
particle amount	-	+/-	++	++	++	-	++
part.size distr.	-	-	+/-	+	+/-	+	+
waterdrops	-	-	-	-	-	+	+
crystals visible	-	-	-	-	-	-	-
sedimentation rate*	+	-	-	--	-	++	--

* sedimentation rate: ++ very fast, -- slow

EXAMPLE 7

Dispersions of reversed vesicles, having the composition as shown in table 5, were prepared according to the method described in example 6. Instead of the reaction vessel a closed vessel, equipped with double walls, was used. The samples were cooled using a cooling bath at 7.5°C. In case ethyl acetate was used as the apolar vehicle the temperature at which the mixture was sonicated was reduced to 60°C. On dispersing the powder of reversed vesicles in Abil® K4, it was observed that the vesicular structure was not changed due to the removal of the apolar vehicle.

Table 5

Reversed vesicle dispersions									
Components									
Sucrose palmitate P-1570	10	10	10	10	10	10	10	10	10
Cholesterol	-	1	2	-	1	2	-	1	2
Water	-	-	-	1	1	1	-	-	-
Isoparaffine (Isopar® E, obtained from Exxon Chemical International)	90	89	88	89	88	87	-	-	-
Ethyl acetate	-	-	-	-	-	-	90	89	88
Mean particle size (nm)	324	471	432	142	120	169	5138	1097	426
Standard deviation	10	15	58	12	8	3	6396	163	104

EXAMPLE 8

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Dispersions of reversed vesicles, having the composition as shown in table 6, were prepared according to the method described in example 7.

After removal of the apolar vehicle 0.5 g of the powder of reversed vesicles was dispersed in 15 g of propylene glycol under stirring at 150 r.p.m. After 6 hours in all batches

maltese crosses could be observed, which is comparable to the results obtained after dispersal of the same product in the silicone oil Abil® K4.

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Table 6

components	%	%	%	%
Sucrose palmitate	10	10	10	10
Cholesterol	1	2	1	1
Water	-	-	0.5	1
Abil® K4	89	88	88.5	88